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Identification and expression of odorant binding proteins in the egg-parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera, Scelionidae, Telenominae)

Kendall King[1], Megan E. Meuti[2], Norman F. Johnson[1,3]

1 *Department of Entomology, The Ohio State University, 1315 Kinnear Road, Columbus, 43212, Ohio, USA* **2** *Department of Entomology, The Ohio State University, Columbus, 43210, Ohio, USA* **3** *Department of Evolution, Ecology & Organismal Biology, The Ohio State University, 1315 Kinnear Road, Columbus, 43212, Ohio, USA*

Corresponding author: Norman F. Johnson (johnson.2@osu.edu)

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Abstract

Trissolcus basalis (Wollaston) (Hymenoptera, Scelionidae) is an egg-parasitoid of the southern green stink bug, *Nezara viridula* (Linneaus) (Hemiptera, Pentatomidae). Many behaviors associated with female *T. basalis* host-finding and acceptance are mediated by chemosensory pathways, for which olfactory, gustatory and ionotropic receptors have been previously identified. Odorant binding proteins (OBPs) are small, globular proteins, one of the functions of which is the transport of odorant ligands through the aqueous lymph of chemosensory sensilla to these receptors. We identified 18 classical OBP sequences in the *T. basalis* genome and transcriptomes sharing an average 26.8% pairwise identity. Gene tree analyses suggest very limited lineage-specific expansion and identify potential orthologs among other scelionids and Hymenoptera. Transcriptome mapping and qPCR comparison of expression levels in antennae and bodies of both sexes determine that at least five TbOBPs are preferentially expressed in the female antennae. These are, therefore, prime candidates for further study to determine their role in detecting host-produced semiochemicals.

Keywords

chemical ecology, chemosenses, expression profiles, OBP, olfaction, Platygastroidea, semiochemicals

Introduction

The subfamily Telenominae (Hymenoptera, Scelionidae) encompasses nearly a thousand species of egg-parasitoids, many of which have proven to be valuable as biological control agents of their host insects (Laumann et al. 2008; Talamas et al. 2015; Sacaccini et al. 2020). Numerous studies have demonstrated that these minute wasps (generally 0.5–2.5 mm in total length) locate eggs to attack by orienting to semiochemicals produced by their adult hosts. These include sex pheromones, alarm pheromones, aggregation pheromones, and cuticular hydrocarbons (Isidoro et al. 2001; Yang et al. 2016). The host specificity of the wasps presumably is mediated by their ability to detect and respond appropriately to these chemicals. In gregarious species (the vast majority), telenomines mate on the host eggs from which the wasps emerge, suggesting that evolution of the chemosensory ability of the female wasp to find eggs may also be a driver of the speciation mechanisms that have resulted in their high species richness (Loch and Walter 2002).

Trissolcus basalis (Wollaston) is a parasitoid of the southern green stink bug, *Nezara viridula* (Linnaeus) (Hemiptera, Pentatomidae). In captivity, the wasps can successfully parasitize other hosts that are presented to them, but data from specimens collected in the field suggest that they specialize on parasitizing *N. viridula* (Johnson 1985). Behavioral experiments have demonstrated that females of *T. basalis* respond to the plant volatiles 3-methylbutanoic acid and p-benzoquinone (Slimani et al. 2016); to the *N. viridula* kairomones (*E*)-2-decenal, 4-oxo-(E)-hexenal, and the sesquiterpenes trans- and cis-(Z)-alpha-bisabolene epoxide; the stink bug cuticular hydrocarbon n-nonadecane; and to mucopolysaccharides on the surface of an egg mass (Bin et al. 1993; Mattiacci et al. 1993; Colazza et al. 2007; Laumann et al. 2009).

The chemosensory mechanisms of *T. basalis* are only beginning to be explored. Previously, we characterized the full repertoire of receptor proteins in this species based on genomic and transcriptomic data: there are 170 olfactory receptors (Ors), 1 copy of Orco, 34 gustatory receptors (Grs), and 23 ionotropic receptors (Irs) (Chen et al. 2021). Several of the Ors are preferentially expressed in the antennae of adult females, suggesting that at least some of these proteins may be tuned to semiochemicals important for host location in the environment. Narrow tuning of the Ors could contribute to the host specificity of this wasp (Andersson et al. 2015).

Before reaching the receptors in the dendritic membrane of the olfactory sensory neurons (OSNs), environmental odorants first interact with another set of proteins, the odorant binding proteins (OBPs). Recent review papers discuss the relevance of OBPs and their role in insect olfaction (Pelosi et al. 2018; Rihani et al. 2021). OBPs may have a generalized function to facilitate the movement of a broad range of hydrophobic odorants to the OSN dendrite through the aqueous sensillar lymph (Vogt et al. 1991; Pelosi et al. 2005; Sun et al. 2016). A number of cases have documented that a subset of OBPs, the pheromone-binding proteins, are themselves narrowly tuned to bind with a small number of compounds (Du and Prestwich 1995; Campanacci et al.

2001; Zhou 2010). OBPs may thus act as an initial filter to limit which odorants can reach and be detected by a specific neuron. Narrow tuning of the response of an OSN may be a function of specificity of the Ors, of the OBPs, or a combination of the two (Andersson et al. 2015).

The objectives of this work were to identify and characterize the OBPs of *T. basalis*, to compare expression levels between males and females, and to determine which, if any, are more highly expressed in the primary olfactory organs, the antennae. The ultimate goal is to integrate information on OBPs with that of the receptor proteins to better understand the physiological mechanisms by which these specialist parasitoids are able to find their hosts in nature.

Materials and methods

Insects

A *Trissolcus basalis* colony originating from the southern U.S.A. was reared on eggs of the spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera, Pentatomidae). The bugs were maintained at 25 °C, 70% relative humidity, and fed a diet of mealworm larvae (*Tenebrio molitor* Linnaeus, Coleoptera: Tenebrionidae). The *Podisus* colony was kept in a non-diapause state using a 12–12 hr light-dark schedule. Mated adult female wasps were removed from the colony and introduced to fresh *Podisus* eggs, and the parasitized eggs were kept separately in vials closed with cotton for roughly 14 days until the adult wasps emerged. Adult wasps were maintained on an alternating diet of simple syrups made from refined white sugar and natural honey. All wasps used for the transcriptome assemblies and gene expression assays were frozen at -80 °C; none of the frozen wasps had been exposed to fresh eggs. Female wasps were frozen the day of their emergence along with any males that may have already emerged.

Protein characterization and gene annotation

OBP amino acid sequences from 14 species across the order Hymenoptera (Table 1) were downloaded from the GenBank non-redundant protein database (https://www.ncbi.nlm.nih.gov/genbank) and used as tblastn queries to search local BLAST databases of the *Trissolcus basalis* transcriptomes (Genbank accession PRJNA625932) and genome (PRJNA49235) (Altschul et al. 1990; Chen et al. 2021). Putative OBP sequences were annotated in the CLC Main Workbench version 21.0.4 and confirmed as OBPs with searches in Pfam version 34.0 (http://pfam.xfam.org/) and Interproscan version 86.0 (https://www.ebi.ac.uk/interpro/). Signal peptide lengths were predicted with SignalP5.0 (http://www.cbs.dtu.dk/services/SignalP), and the molecular weights of each protein were predicted using BioInformatics calculator (https://www.bioinformatics.org/sms/index.html).

Table 1. List of taxa of OBPs used to query *Trissolcus basalis* transcriptome and genome local BLAST databases.

Family	Species	No. of OBPs from GenBank
Cephidae	*Cephus cinctus*	26
Orussidae	*Orussus abietinus*	5
Bethylidae	*Sclerodermus* sp.	10
Formicidae	*Solenopsis richteri*	8
Apidae	*Apis cerana*	27
Apidae	*Apis mellifera*	34
Braconidae	*Aphidius gifuensis*	11
Braconidae	*Diachasma alloeum*	16
Braconidae	*Microplitis mediator*	7
Aphelinidae	*Encarsia formosa*	39
Encyrtidae	*Copidosoma floridanum*	6
Pteromalidae	*Nasonia vitripennis*	84
Trichogrammatidae	*Trichogramma japonicum*	9
Scelionidae	*Telenomus podisi*	3
Scelionidae	*Trissolcus japonicus*	3

Taxanomic sources of OBPs used to search for potential OBP sequences in the genome of *Trissolcus basalis.*

Gene tree analysis

A total of 136 OBP sequences for *Trissolcus basalis*, *Cephus cinctus* Norton (Cephidae; Robertson et al. 2018), *Apis mellifera* Linnaeus (Apidae) (Forêt and Maleszka 2006), *Nasonia vitripennis* (Walker)(Pteromalidae)(Vieira et al. 2012), *Trissolcus japonicus* (Ashmead) and *Telenomus podisi* Ashmead (Farias et al. 2015) were aligned with MUSCLE version 3.8.31 (Edgar 2004) in MegaX version 11.0 (https://www.megasoftware.net/) using the default settings. Accession numbers for each protein included in the analysis may be found in Suppl. material 1 in the HymOBP tab. OBPs of different species are distinguished by prefixing the string OBP## with the first letters of the genus and species name of each taxon: Tb, Cc, Am, Nv, Tj, or Tp. The relationships among the aligned sequences were then analyzed in a maximum likelihood context in MegaX using the amino acid substitution model LG+G (Le and Gascuel 2008, Tamura et al. 2013) based on MegaX's best-fit analysis, with 1000 bootstrap replicates. CcOBP12 was chosen as the root based on a nearest neighbor analysis of OBPs of *Cephus cinctus*, the outgroup.

Measurement of OBP expression levels

Preliminary relative expression levels were made with Geneious 20.2.3 (https://www.geneious.com) by mapping RNA-seq reads to the TbOBP sequences for all transcriptomes. Those results were followed up by measuring expression levels using quantitative real-time PCR (qPCR).

Antennae were removed from the bodies from 500 male and 500 female specimens using fine-tipped forceps. These were divided into five biological replicates for each combination of sex and tissue: male antennae (100 antennae), female antennae (100 antennae), male bodies (five bodies), and female bodies (five bodies). RNA was

extracted using 500 µL TRIzol Reagent following the manufacturer's protocol and purified using phenol-chloroform. RNA concentration (0.3–3.0 µL) was measured using Qubit RNA HS Assay Kits, and 20 µL cDNA was synthesized from input of 92 pg of RNA using ThermoScientific Maxima First Strand cDNA Synthesis Kit according to the manufacturer's instructions and diluted in 80 µL H_2O for qPCR.

Two pairs of primers were designed for each OBP using Primer3Plus v2.4.2 (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Each primer set is 20 bp in length with a melting temperature of 60±0.3 °C (Table 2). Primer specificity and performance were confirmed using melt and standard curves to ensure that each primer set met MIQE guidelines (Bustin et al. 2009). The relatively short sequences for these proteins limited the possibilities for more primer design. Primers for seven TbOBP nucleotide sequences – TbOBP5–9, TbOBP12, TbOBP15 – did not fall within a tested efficiency range between 80–110 %, and no results for those are presented. Features of the primers used in the experiments are presented in Suppl. material 1: qPCR Primers tab.

Relative expression levels were measured using BioRad CFX qPCR instruments and CFX Maestro software 1.1 (https://www.bio-rad.com). In brief, all reactions were run in triplicate, each in a total volume of 10 µl containing 5 µL of iTaq Universal SYBR Green Supermix (BioRad), 400 nm of primer, and 1 µL of cDNA on a 96-well plate. The relative cycle threshold (CT) of the technical replicates was averaged for each biological replicate. The resulting CT value for each OBP was normalized to the geometric average of the CT values of three reference genes RpL32 (ribosomal protein L32), RpL19 (ribosomal protein L19), and the nuclear ribosomal gene 18S by subtracting the geometric average CT of reference genes for each biological replicate from the CT value for the gene of interest within that biological replicate using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Statistical analysis

All statistical analyses were done in R version 4.0.5 (https://www.R-project.org/). Statistical significance in the mean expression level of each tissue type relative to others was determined with an ANOVA at an alpha value of 0.05 for eleven TbOBPs. Mean separations were assessed with Tukey's HSD test.

Results

Identification and classification of *Trissolcus basalis* OBPs

Eighteen TbOBP sequences were identified, ranging in length from 290–591 bp (96–197 aa), and having an average pairwise amino acid identity of 26.8% (Table 2, Suppl. material 1: 1: TbOBPs tab). Sixteen of these sequences were identified as belonging to the PB_GOBP family in Pfam searches; TbOBP6 and TbOBP7 were not assigned to

Table 2. Odorant binding proteins of *Trissolcus basalis*.

Gene name	GenBank accession	Molecular Weight (kD)	Signal peptide	Scaffold: position	Number of exons	Length (bp)
TbOBP1	MZ488428	16.15	1–20	scaffold_1144: 9533–10939	5	438
TbOBP2	MZ488429	15.82	N/A	scaffold_102: 26609–25696	4	396
TbOBP3	MZ488430	14.56	1–17	scaffold_1037: 13416–15382	5	405
TbOBP4	MZ488431	16.16	1–21	scaffold_159: 35484–34592	6	390
TbOBP5	MZ488432	16.39	1–22	scaffold_159: 41468–40636	6	432
TbOBP6	MZ488433	16.04	1–21	scaffold_4916: 3562–2759	5	420
TbOBP7	MZ488434	18.53	1–20	scaffold_353: 17641–18623	5	498
TbOBP8	MZ488435	16.91	1–16	scaffold_353: 21945–21153	5	446
TbOBP9	MZ488436	15.17	1–19	scaffold_159: 39147–37845	5	411
TbOBP10	MZ488437	15.61	1–20	scaffold_353: 19949–19112	5	417
TbOBP11	MZ488438	14.5	1–20	scaffold_353: 20230–20931	5	390
TbOBP12	MZ488439	15.47	1–22	scaffold_1177: 26917–26077	5	423
TbOBP13	MZ488440	15.2	1–19	scaffold_165: 6291–5476	5	390
TbOBP14	MZ488441	15.76	1–20	scaffold_353: 23655–24502	5	420
TbOBP15	MZ488442	22.71	1–30	scaffold_32: 232386–231315	6	591
TbOBP16	MZ488442	17.17	1–20	scaffold_159: 42798–41880	7	468
TbOBP17	MZ488444	17.14	1–20	scaffold_496: 26802–28032	6	465
TbOBP18	MZ488445	18.96	1–21	scaffold_79: 22955–21677	5	489

Characteristics of odorant binding protein genes of *Trissolcus basalis*.

family in the Pfam query but were characterized as belonging to the PB_GOP superfamily in InterProScan. All TbOBPs exhibit the Classical OBP pattern of C1-X_{15-39}-C2-X_{3}-C3-X_{21-44}-C4-X_{7-12}-C5-X_{8}-C6 (Bohbot and Vogt 2005) with six conserved cysteine residues. The molecular weights range from 14.5–22.7 kD. Although both atypical OBPs and C-plus OBPs (Xu et al. 2003; Spinelli et al. 2012) were used as queries, none of the sequences found in the *T. basalis* genome or transcriptomes display those motifs. Signal peptides were measured within the first 30 bp of each TbOBP with the exception of TbOBP2, for which no signal peptide or cleavage site was detected. Nine of the TbOBPs were found on just two genome scaffolds (Fig. 1). Scaffold_159 is 79,446 bp in length and contains TbOBP9, TbOBP5, TbOBP16, and TbOBP4 within an extent of 4,555 bp. Scaffold_353 is 86,326 bp in length and contains TbOBP7, TbOBP10, TbOBP11, TbOBP8, and TbOBP14 in a span of 6,861 bp. Only one protein-coding gene was found between any two of these TbOBP sequences within the same scaffold: between TbOBP8 and TbOBP14 on scaffold_353 is gene TRIBAS_03633 of unknown family and function.

Phylogenetic analysis of Hymenoptera OBPs

The results of the maximum likelihood analysis of the relationship between OBPs in *Trissolcus basalis* and those of *Cephus cinctus*, *Nasonia vitripennis*, *Apis mellifera*, and the few OBPs so far characterized for the telenomines *Trissolcus japonicus* and *Telenomus podisi* are presented in Fig. 2. The low boostrap values in some internal nodes resulted from low similarities among the analyzed OBP sequences. TbOBP6, TbOBP7, and TbOBP14 form a small lineage-specific expansion in *T. basalis* (98% support). Simi-

Figure 1. Position and orientation of syntenic TbOBPs present on genomic scaffolds 159 and 353. Yellow connected ribbon segments represent exon regions of each TbOBP. The black vertical lines labeled with gene names are located at the 5' end of each sequence.

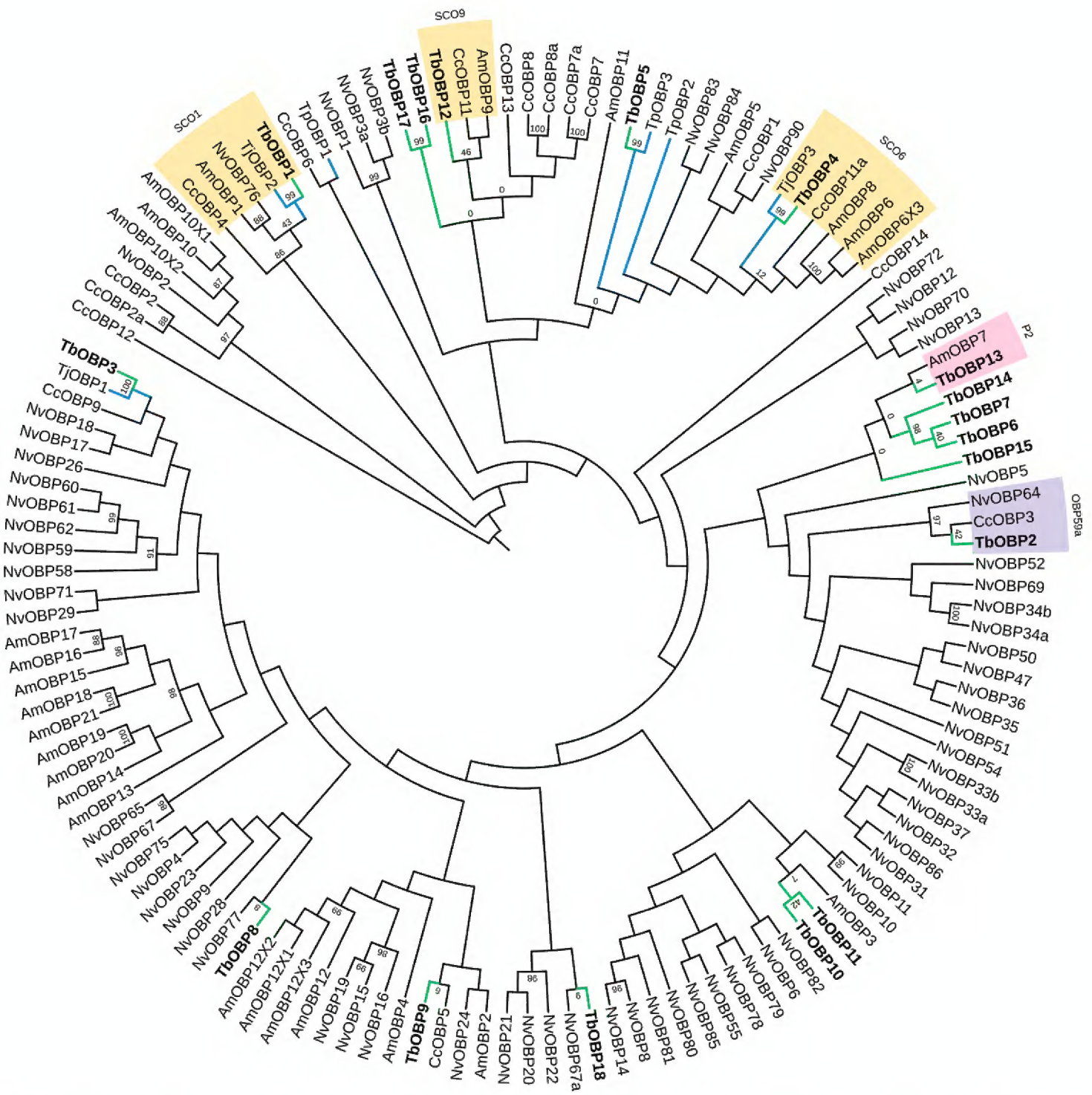

Figure 2. Maximum likelihood tree illustrating relationships among 136 OBP sequences from *Nasonia vitripennis* (Nv), *Apis mellifera* (Am), *Cephus cinctus* (Cc), *Trissolcus japonicus* (Tj), *Telenomus podisi* (Tp), and *Trissolcus basalis* (Tb). The tree is rooted with CcOBP12. TbOBPs are depicted in bold text and branches highlighted in green, other telenomine species are highlighted in blue. TbOBPs that cluster with single-copy orthologs (SCO), paralog group 2 (P2), and the OBP59a group of McKenzie et al. (2014) are highlighted in yellow, pink, and purple respectively. All bootstrap support values ≥ 85% are indicated at the relevant nodes; lower values are reported to clarify support level at other selected nodes.

larly, TbOBP16 and TbOBP17 cluster together as a pair (99% support). Possible orthologs with the other telenomines are TbOBP3 and TjOBP1 (100%); TbOBP4 and TjOBP3 (99%); and TbOBP5 and TpOBP3 (99%). TbOBP1 and TjOBP2 (99%) group together along with possible orthologs in *Cephus*, *Nasonia* and *Apis* (86% bootstrap support). Beyond its immediate close relatives in the Telenominae, TbOBP2 groups together with NvOBP64 and CcOBP3 (97%).

Expression of TbOBPs

The number of RNA-seq in each of the four transcriptomes were mapped to the TbOBP sequences as initial measurements of relative expression levels (Table 3). Expression levels are reported as Normalized TPM (transcripts per million, Wagner et al. 2012). Four OBPs are expressed at higher levels in the male antenna than the female antenna: TbOBP1, TbOBP4, TbOBP5, and TbOBP6 ($\log_2$ ratio of expression levels > 2). Eight OBPs are expressed at higher levels in the female antenna than the male antenna: TbOBP8, TbOBP10–13, TbOBP15–17. In the rest of the body, male-biased expression levels are suggested in TbOBP4–5, and female-biased expression in TbOBP10–11 and TbOBP16–17. The TbOBPs with sex-biased expression levels in the body also show the same bias in the antennae.

The transcriptome mapping represents only a single sample of OBP expression levels. Therefore, we used qPCR to confirm that the patterns of expression identified in the transcriptome were consistent in multiple, independent samples.

Table 3. Mapping of transcriptome reads to TbOBP sequences.

Gene	Normalized TPM				♂/♀ Ratio ($\log_2$)	
	FA	MA	FB	MB	Antenna	Body
TbOBP1	37320.61	218172.8	814.31	2380.81	2.55	1.55
TbOBP2	16413.96	19051.54	351804.3	228136.8	0.21	-0.62
TbOBP3	313.56	465.64	13810.42	9442.63	0.57	-0.55
TbOBP4	15474.3	64711.87	7714.45	38576.66	2.06	2.32
TbOBP5	884.06	5663.17	5.62	32.07	2.68	2.51
TbOBP6	9341.61	29195.28	10075	10901.98	1.64	0.11
TbOBP7	27516.63	2332543	417526.7	543738.2	3.08	0.38
TbOBP8	126899.5	1510.92	3215.15	2415.79	-6.39	-0.41
TbOBP9	9551.89	9551.9	84336.73	77414.59	0	-0.12
TbOBP10	17278.82	205.73	738.95	318.47	-6.39	-1.21
TbOBP11	16857.74	165.63	429.27	103.64	-6.67	-2.05
TbOBP12	28.64	20.1	17.2	3344.31	-2.11	7.6
TbOBP13	7260.78	23.43	111.99	29.61	-8.28	-1.92
TbOBP14	84488.98	113486.4	57463.33	101428.3	0.73	0.82
TbOBP15	101067	4035.22	4162.92	4955.34	-4.65	0.25
TbOBP16	429238.4	1467.28	6210.96	323.25	-8.19	-4.26
TbOBP17	524941.4	1504.04	7367.66	511.59	-8.45	-3.85
TbOBP18	0	11.42	5046.14	5970.07	–	0.24

Mapping of transcriptome reads to each of the TbOBP sequences. TPM: transcripts per million; FA: female antennal transcriptome; FB: female body transcriptome; MA: male antennal transcriptome; MB: male body transcriptome. Positive numbers in the male/female ratio indicate male-biased expression, negative numbers indicate female-biased expression. The ratio for the antennae of TbOBP18 is undefined due to the absence of any reads mapped to the female antenna.

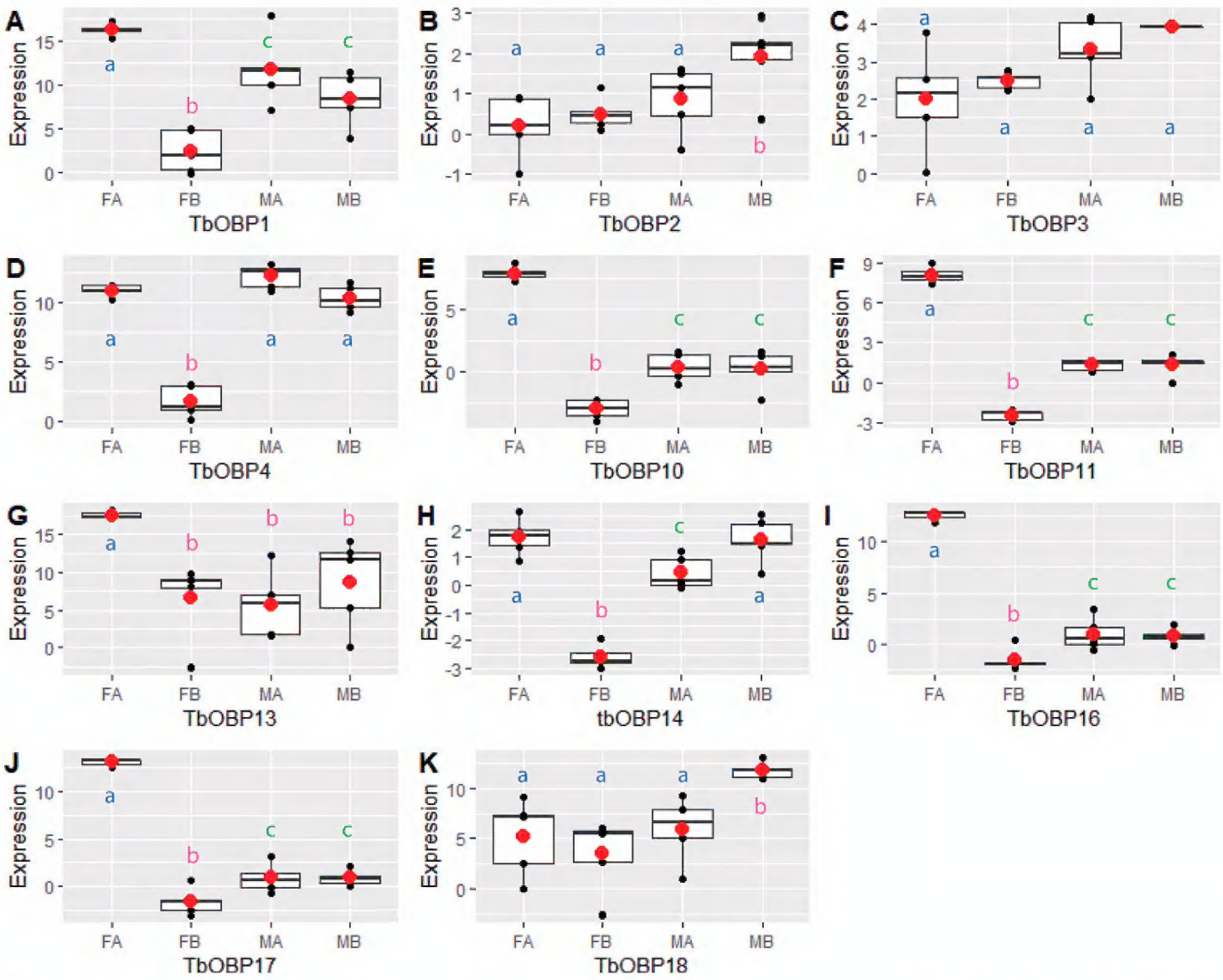

Figure 3. Expression profiles for 11 TbOBPs across four tissue types as measured with qPCR: female antennae (FA), female bodies (FB), male antennae (MA), and male bodies (MB). Black data points: normalized expression (log2) for each of five biological replicates per tissue; red dots: mean expression level. Significance differences ($p < 0.05$) are illustrated by differences in letters, calculated using TukeyHSD ANOVA means comparison.

Expression levels of individual TbOBP genes are presented in Fig. 3. Six of the 11 OBPs show female-biased antennal expression and were significantly more abundant in female antennae than any other tissue: TbOBP1, TbOBP10–11, TbOBP13, and TbOBP16–17. There was no male-biased expression of OBPs in the antennae. Seven OPBs are expressed at higher levels in the male bodies than in the female bodies: TbOBP1, TbOBP4, TbOBP10–11, TbOBP14, TbOBP16–17. None of the qPCR experiments showed female-biased body expression.

Discussion

The repertoire size of OBP-coding genes varies across characterized Hymenoptera genomes, from 14 genes in *Linepithema humile* to 90 in *Nasonia vitripennis* (Vieira et al. 2012; McKenzie et al. 2014). Among insects, the number of OBP-coding genes ranges

widely, with over 100 OBPs reported from both of the mosquitoes *Culex quinquefasciatus* and *Aedes aegypti* (Manoharan et al. 2013; Rehani et al. 2021). The repertoire of 18 Classical OBPs we found in the genome of *Trissolcus basalis*, therefore, is on the low end of this spectrum. There are several different motifs among OBPs; beyond the Classical OBPs, non-classical motifs include dimer-OBPs with two sets of 6 conserved cysteines, C-plus with 8 conserved cysteines, C-minus with 4 conserved cysteines, and Atypical with 9–10 cysteines and a long terminus (Zhou 2010). Although it is common to find OBPs that fall within non-classical motifs, it is not unusual to find only sequences with the six conserved cysteine sites consistent with the Classical OBP motif (Gu et al. 2011; Lagarde et al. 2011; Zheng et al. 2016).

The small number of OBPs in *Trissolcus basalis* limits the possible number and size of lineage-specific expansions in this species. Among the 18 TbOBPs and based on high bootstrap values, we have confidence for the existence of only one pair and one trio of genes. Therefore, in contrast with a species such as *Nasonia vitripennis*, evolution in the number of OBPs in *Trissolcus* has been fairly conservative. This is also apparent in our gene tree analysis, which resulted in few lineage-specific expansions among *T. basalis* OBPs.

McKenzie et al. (2014) recognized four groups of OBPs from ants that they named the single-copy ortholog group, paralog group 1, paralog group 2, and the OBP59a group. They concluded that the two paralog groups were specific to aculeate Hymenoptera. A grouping of *Trissolcus* OBPs with one member of paralog group 2, AmOBP7, is suggested by the topology of the tree in Fig. 2. The bootstrap values for the nodes linking TbOBP14, TbOBP7, and TbOBP6 to that *Apis* protein have 0% bootstrap support, indicating that this clustering is spurious. The 4% bootstrap support for the node linking AmOBP7 and TbOBP13 is extremely low and may indicate that this association is also trivial. McKenzie et al. (2014) also reported that the *Nasonia* genome encodes a gene in the OBP59a group, and on the basis of their tree of hymenopteran OBPs in the supplemental information, we concluded that this was NvOBP64. A key finding in our analysis is that we found that TbOBP2 groups with NvOBP64 (97%), and the two are likely orthologs. Finally, each of the six lineages of OBPs in the single-copy ortholog group identified by McKenzie et al. (2014) were reported to have counterparts in *Nasonia* as well as in other groups outside of Hymenoptera. Therefore, they concluded that these genes probably date back at least to the origin of Holometabola. In our data, however, three of these lineages –OBP5, OBP10, OBP11 – have no clear ortholog in *T. basalis*. Of the three remaining, TbOBP1 clusters with AmOBP1 (43%); TbOBP4 with AmOBP6 (12%); and TbOBP12 with AmOBP9 and CcOBP11 (46%). The "single-copy orthologs" may be ancient as suggested by McKenzie et al. (2014), but our data suggest that not all of them have been retained in a recognizable form in the Scelionidae.

Our measures of relative expression levels in tissues and sexes differed in a few cases between transcriptome mapping and qPCR experiments. Only one transcriptome for each combination of sex and tissue was sequenced, and a mapping of transcriptome reads for each such combination is, therefore, only a single point estimate of the true

expression level. In contrast, the qPCR experimental levels were calculated on the basis on five biological samples. We have greater confidence in the qPCR results, and some of the values derived from the mappings may be individual extremes. It is also possible that, despite our efforts to maintain constant rearing conditions, the conditions experienced by the wasps prior to collection may have differed in some manner that affected gene expression.

All OBPs found in the genome of *T. basalis* were recovered in at least one of the adult transcriptomes. This suggests that no OBPs are expressed solely in the immature stages of these parasitoids. Insofar as at least some these proteins are involved in chemosensation, this is not surprising. The larvae of *T. basalis* develop within the eggs of their hosts, a relatively homogeneous and isolated environment. We would anticipate, though, that in their more generalized function as encapsulins or transporters of hydrophobic ligands, OBPs would still play an important role in nutrition and development in all instars of the wasps (Ishida et al. 2013).

Our motivation in this work is to better understand the chemosensory role of OBPs. Therefore, our attention was focused on those proteins expressed at higher levels in the antennae, the structure that has been demonstrated to be necessary for host-finding and acceptance (Safavi 1968). Transcriptome mapping suggested four male-biased antennal genes: TbOBP1, TbOBP4, TbOBP5, and TbOBP7. However, qPCR measurements resulted in no evidence for higher expression of any OBP in the male antennae. TbOBP1 was, in fact, expressed at significantly higher levels in the female antennae, and TbOBP4 was expressed at relatively high levels (in comparison with the bodies) in the antennae of both sexes. TbOBP5 and TbOBP7 could not be measured by qPCR. The initial transcriptome mapping suggested eight genes preferentially expressed in the female antenna: TbOBP8, TbOBP10–13, and TbOBP15–17. Measurements using qPCR confirmed this expression pattern for TbOBP10–11, TbOBP13, and TbOBP16–17. TbOBP8, TbOBP12, and TbOBP15 could not be measured with qPCR. Genes TbOBP16 and TbOBP17 group together in our gene tree analysis but are found on different scaffolds, suggesting that they did not arise from a simple gene duplication. On scaffold_159, TbOBP16 is flanked on either side by TbOBP4 and TbOBP5, further isolating it from TbOBP17. In contrast, TbOBP8, TbOBP10 and TbOBP11 are syntenic on scaffold_353. TbOBP10 and TbOBP11 also group together on the gene tree and have similar expression profiles, possibly suggesting a common regulatory element.

Up-regulation of a subset of TbOBPs in the female antennae suggests to us that the proteins play a chemosensory role in mediating female-specific behaviors. These may include mate recognition, host habitat recognition, long-range host finding, short-range host acceptance, and recognition of marking pheromones applied after oviposition (Salerno et al. 2017; Slimani et al. 2017; Foti et al. 2019). With the data available, it is still unclear what role these proteins might play in *T. basalis*. We would predict that TbOBP1 has some general function in olfaction. We base this conjecture on the conservation of this gene across all Hymenoptera. Salerno et al. (2012) identified components of a female-produced sex pheromone in the species *Trissolcus brochymenae*

(Ashmead) that stimulates intense antennation and mounting by males. Notably, we found no indication in the qPCR experiments of any TbOBP that is more highly expressed in the male antenna. We recognize that functionally important olfactory proteins may be expressed in low levels, and that high expression is not a requirement to be important and functional.

McKenzie et al. (2014) concluded that, in ants, the OBPs were "likely involved in conserved aspects of olfaction" and that the OBPs "may not rapidly evolve to recognize species-specific signals" (emphasis added). We need more information on the OBPs in other, related telenomines to be able to assess the birth and death rates of these genes and whether a subset of them have been similarly conserved in the evolution of the subfamily.

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Supplementary material 1

TbOBP sequences; OBP sequences for gene tree; qPCR primers

Authors: Kendall King, Megan E. Meuti, Norman F. Johnson

Data type: protein sequences, Genbank acccession numbers, primer sequences

Explanation note: TbOBPs tab: amino acid sequences of *Trissolcus basalis* odorant binding proteins. HymOBPs tab: GenBank accession numbers of hymenopteran odorant binding proteins used in gene tree analysis. qPCR primers: primer sequences used in qPCR measurements of expression levels of *Trissolcus basalis* olfactory binding proteins.

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